Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. 200461036

yDNA: a New Geometry for Size-expanded Base Pairs

Haige Lu, Kaizhang He,[Ψ] and Eric T. Kool*

[*] Prof. Dr. E. T. Kool, H. Lu
Department of Chemistry, Stanford University
Stanford, CA 94305-5080 (USA)
Fax: (+1) 650-725-0259
E-mail: kool@leland.stanford.edu

[Ψ] Dr. K. He
Current address: Pierce Biochemical Co., Milwaukee, WI 53202

CONTENTS

General. Chemicals and solvents were either purchased from commercial suppliers or purified by standard methods. For thin-layer chromatography (TLC), EM Science Silica Gel 60 F_{254} plates were used and compounds were visualized by irradiation with UV light. Silica column chromatography was performed using Selecto Scientific Silica Gel, sizes 32-63. All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 instrument. $CDCl_3$ and CD_3OD were used as NMR solvents as well as internal standards for ^{1}H and ^{13}C NMR. High-resolution mass spectra were taken at the University of California at Riverside Mass Spectrometry Facility (UCRMS), Riverside, California. HPLC analysis and purification was carried out on a Shimadzu HPLC instrument consisting of an SPD-M10A detector, an SCL-10A system controller and an LC-10AD liquid chromatography with reverse- phase C_{18} columns. A Hypersil BDS column was used for analysis and a ZORBAX ODS column was used for preparation.

Synthesis and characterization of dyA phosphoramidite

5-methyl-4-nitroindole (2) To 4.86 g 4-nitroindole(30.0 mmol) was added 270 mL anhydrous THF and 12 mL HMPA. The solution was cooled to −30 °C by salt-ice bath. To the mixture was added 30 mL 3 M methylmagnesium chloride(90 mmol) slowly. The dark solution was stirred for another 15 min before a solution containing 14.8 g $Pb(OAc)_4$ (33.3 mmol), 110 mL CH_2Cl_2 and 1.8mL glacial acetic acid(31 mmol) was poured in. The mixture was left in the bath for 5 min before being moved out and stirred at room temperature for 1 h. 7.3 mL ethylene glycol was added and the suspension was stirred another 1 h. The suspension was then filtered and the crude product was separated by silica gel chromatography (hexane/ethylacetate =4/1) to afford 6.2 g of a yellow solid mixture of 5-methyl-4-nitroindole and 7-methyl-4-nitroindole with a ratio of 5:2. Total yield for 5-nitroindole was 42%. The regioisomers were carried to the next step without further purification. Pure 5-methyl-4-nitro indole was, however, obtained by deprotection of 5-methyl-4-nitro-1-phenyl-sulfonylindole. The structure of the desired 5-isomer was identified by HMBC NMR method(data not shown).

^{1}H NMR($CDCl_3$,500 MHz): δ 8.59(s,1H),7.53 (d,1H,J=10 Hz),7.37(m,1H), 7.10(d,1H,J=10 Hz), 6.95(m,1H),2.68(s,3H). ^{13}C NMR($CDCl_3$,125 MHz): δ 141.02, 135.95, 127.44, 126.61, 125.61, 122.73, 115.95, 102.33, 20.43. HRMS(DEI): calcd. for $C_9H_8N_2O_2$(M^+) 176.0586; found 176.0579.

5-Methyl-4-nitro-1-phenylsulfonylindole (3) 5.8 g 5-methy-4-nitroindole and 7-methylindole 5:2 mixture (35 mmol total methylnitroindole) and 2.0 g NaOH pellets (52 mmol) were added to a flask. To this was added 65 mL CH_2Cl_2. The mixture was stirred at room temperature for 30 min. 6.3 mL $PhSO_2Cl$ was then added dropwise. The suspension was stirred at room temperature under inert atmosphere for 20 h and was filtered and the filtrate was set aside. The precipitate on filter paper was washed intensively by water followed by ether. The organic layer of the filtrate was concentrated. Some precipitate appeared and was then filtered, washed by water and ether. Both precipitates were collected and dried under vacuum and P_2O_5 to give 6.4 g 5-methyl-4-nitro-1-phenylsulfonylindole in 87% yield. ^{1}H NMR($CDCl_3$,500 MHz): δ 8.09(d,1H,J=10 MHz),7.86(m, 2H),7.68(d,1H,J=3.5 MHz),7.56(m,1H), 7.46(m,2H),7.23(d,1H, J=10 MHz),7.01(m,1H),2.59(s,3H). ^{13}C NMR ($CDCl_3$,125 MHz): δ 141.60,137.67,134.39,134.34,129.53,129.18, 129.00,128.43,126.76,125.62,117.56,107.6,19.91. HRMS(DEI): calcd. for $C_{15}H_{12}N_2O_4S$(M^+) 316.0518; found 316.0505.

4-Nitro-1-phenyl-sulfonyl-5-indolecarboxaldehyde (4) To 6.0 g N-phenylsulfonyl-5-methyl-4-nitroindole (19.0 mmol) was added 300 mL anhydrous DMF. The suspension was then heated to 105 °C. To this was then added 3.9 mL $HC(NMe_2)_3$ (22.8 mmol). The dark red solution was stirred at 105 °C under Ar atmosphere for 3 h. The mixture was then concentrated under reduced pressure. To the dark red oily residue was added 380 mL anhydrous THF followed by 4.5 g $KMnO_4$ (28.5 mmol) in 38 mL water. The mixture was stirred at room temperature for 3 h. It was then filtered and washed by EtOAc followed by brine. The crude product was purified by silica gel chromatography (dichloromethane/methanol=100/1) to afford 7.6 g of 4-nitro-

1-phenylsulfonyl-5-indole-carboxaldehyde in 70% isolated yield. ^{1}H NMR($CDCl_3$,500 MHz): δ 10.33(s,1H),8.33(d,1H),7.89(m,3H), 7.86(d,1H,J=3.5 Hz),7.62(m,1H),7.50(m,2H), 7.18(d,1H,J=4.5 Hz). ^{13}C NMR ($CDCl_3$,125 MHz): δ 187.57,143.01,138.48,137.29,134.92, 131.15, 130.30,130.28,129.82,126.93,125.05,124.87,118.09,107.68. HRMS (DEI): calcd. for $C_{15}H_{10}N_2O_3S(M^+)$ 330.0310; found 330.0306.

4-Amino-1-phenyl-sulfonyl-5-indolecarboxaldehyde (5) 7.6 g 4-nitro-1-phenylsulfonyl-5-indolecarboxaldehyde (23.0 mmol) was dissolved in 600 mL THF. To it was added 23.6 g $Na_2S_2O_4$(technical grade, 85% purity, 116 mmol) in 300 mL H_2O. The mixture was stirred at 50 °C under N_2 for one hour. The organic layer was separated and dried with Na_2SO_4. It was finally concentrated to afford 6.5 g red foam. The crude product was used for the next reaction without further purification. However, it could be purified by silica gel column chromatography (Hexane/Ethylacetate=4/1). ^{1}H NMR ($CDCl_3$,500 MHz): δ 9.85(s,1H), 7.88(m,2H),7.56(m,1H),7.50(d,1H,J=3.5 Hz),7.46(m,2H),7.37(s,2H), 6.65(d,1H,J=3.5 Hz),6.57(br s,2H). ^{13}C NMR($CDCl_3$,125 MHz): δ 193.35, 144.83, 138.33, 137.82, 134.18, 132.118, 129.38, 126.78, 124.75, 117.93,113.32,105.62,102.80. HRMS(DEI): calcd. for $C_{15}H_{12}N_2O_3S(M^+)$ 300.0569; found 300.0570.

5-amino-1H-pyrrolo[2,3-f]quinazoline (6) 6.5 g Crude 4-amino-1-phenyl-sulfonyl-5-indolecarboxaldehyde was dissolved in 100 mL anhydrous N,N-dimethylacetamide. To this solution was then added 7.8 g (43 mmol) guanidine carbonate and argon was bubbled through the mixture at room temperature for 30 min. Fit with a condenser, the mixture was immersed in a pre-heated oil bath at 150 °C and kept stirring under Ar atmosphere for 4 h before being cooled to room temperature. The dark mixture was filtered. The precipitate was washed by ethylacetate, followed by water and ether. 2.6 g pale white powder was obtained after drying under vacuum and P_2O_5 in 61% yield in two steps. ^{1}H NMR(DMSO,500 MHz): δ 11.60(s,1H), 8.93(s,1H),7.38(d,1H,J=8.5 Hz),7.34(t,1H,J=2.5 Hz),7.29(d,1H, J=8.5 Hz),6.88(t,1H,J=2 Hz),6.65(s, 2H). ^{13}C NMR(DMSO,125 MHz): δ

161.09,160.56,148.50,137.61,123.39,120.77,120.27,114.14,109.82, 101.76. HRMS(DEI): calcd. for $C_{10}H_8N_4$(M^+) 184.0749; found 184.0744.

1-[2′-Deoxy-3′,5′-di-O-toluoyl-β-D-ribofuranosyl]-5-[N-(dimethylamino)methylidine]amino-pyrrolo[2,3-f]quinazoline (7) 1.15 g 5-amino-1H-pyrrolo[2,3-f]quinazoline (6.25 mmol) was co-evaporated with toluene twice before being mixed with 316 mg 95% dry NaH powder (12.5 mmol). To the mixture at room temperature was added 250 mL anhydrous acetonitrile. The suspension was stirred under Ar atmosphere at 0 °C for 30 min. 3.65 g Hoffer's chlorosugar (9.4 mmol) (M. Hoffer, *Chem. Ber.* **1960**, *93*, 2777-2781) was added in one portion. The mixture was stirred at 0 °C for 90 min and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (dichloromethane/methanol =100/1, 1% TEA) to afford 3.08 g slight orange solid in 92% yield with an epimer ratio of α:β>20:1. The structure identification of α and β isomers were achieved by 1D NOE NMR method (data not shown). ^{1}H NMR($CDCl_3$,500 MHz): δ 8.92(s,1H),7.97(d,2H,J=8 Hz),7.90(d,2H, J=8 Hz), 7.46(d,1H,J=9 Hz), 7.35(m,2H), 7.28(m,2H), 7.23(m,2H), 7.16(d,1H,J=3 Hz),6.51(dd,1H,J=8.5,5.5 Hz),5.72(m,1H),4.66(m, 2H),4.60(m,1H),2.87(m,1H), 2.71(m,1H),2.43(s,3H),2.40(s,3H). ^{13}C NMR($CDCl_3$,125 MHz): δ 166.20,165.97,161.12,160.56,148.53, 144.49,144.10,137.80,129.75,129.63,129.27,129.23,126.68,126.42 ,122.56,122.23, 121.73,115.54, 108.87,103.80,85.64, 81.99,74.92, 64.06, 38.30, 21.73, 21.67. HRMS(FAB): calcd. for $C_{31}H_{29}N_4O_5$($M+H^+$) 537.2137; found 537,2124.

1-[2′-Deoxy-β-D-ribo-furanosyl]-5-amino-pyrrolo[2,3-f]-quinazoline (8) 3.08 g protected 2′-deoxyriboside **7** (5.74 mmol) was dissolved in 140 mL anhydrous dichloromethane. 34.4 mL 0.5 M NaOMe in methanol (17.2 mmol) was quickly added into the solution. The solution was stirred at room temperature under Ar atmosphere for 140 min. and was then filtered. The precipitate was collected and dried under vacuum to yield 0.90 g product in white powder. The

filtrate was concentrated and purified by silica gel column chromatography (dichloromethane/methanol=8/1) to afford 0.66 g slight yellowish powder. Combined products gave a total isolated yield of 91%. ^{1}H NMR(CD_3OD,500 MHz): δ 8.96(s,1H),7.57(m,2H), 7.50(d,1H,J= 8.5 Hz),7.14(d, 1H,J=3.5 Hz),6.52(dd,1H,J=6.5,7.5 Hz),4.51(m,1H),3.98(m, 1H),3.72(m,2H), 2.62(m,1H),2.40(m,1H). ^{13}C NMR(CD_3OD,125 MHz): δ 162.44, 161.76,149.18,139.81,124.58, 122.63, 122.35, 116.27, 110.30, 103.98, 88.38, 86.24, 72.56, 63.33,41.44. HRMS(DEI): calcd. for $C_{15}H_{16}N_4O_3$(M^+) 300.1222; found 300.1230.

1-[2′-Deoxy-5′-O-(4,4′-dimethyloxytrityl)-β-D-ribofuranosyl]-5-[N-(dimethylamino)methylidine]amino-pyrrolo-[2,3-f]Quinazoline (9) 680 mg free 2′-deoxyriboside (2.27 mmol) was suspended in 23 mL anhydrous pyridine and 3.0 mL dimethyl-formamide dimethylacetal (22.7 mmol). The mixture was stirred under Ar atmosphere at 40 °C for 16 h. Most of the solvent was then evaporated under reduced pressure. To the concentrated mixture was then added 23 mL anhydrous pyridine and 0.51 mL Diisopropylethylamine (3.0 mmol). 1.15 g 4,4′-dimethoxy- trityl chloride (3.4 mmol) was added subsequently in a single portion. The mixture was stirred at room temperature under Ar atmosphere for one hour before concentrated *in vacuo*. The residue was dissolved in 50 mL dichloromethane and washed by saturated sodium bicarbonate twice and dried over sodium sulfate. The crude product was purified by silica gel column chromatography (dichloromethane/methanol= 50/1, solvents saturated by ammonia gas) to give 967 mg slight brown foam in 62% overall yield in two steps. ^{1}H NMR($CDCl_3$,500 MHz): δ 9.07(s,1H),8.85(s,1H),7.55(d,1H,J=9 Hz),7.39(m,3H),7.29-7.16 (8H),6.73(m,4H),6.46(t,1H,J=6.5 Hz),4.71(m,1H),4.17(m,1H), 3.71 (s,6H),3.34(m,2H), 3.18(s,3H), 3.15(s,3H), 2.61(m,1H),2.50(m,1H). ^{13}C NMR($CDCl_3$,125 MHz):δ 163.53,160.30, 158.39,148.57, 144.58, 137.51, 135.69, 135.63, 130.02, 128.11, 127.78,126.79, 123.07, 122.97, 120.92, 117.40, 113.04, 110.71, 103.83, 86.38, 85.44, 85.09, 72.22, 64.03, 41.09, 40.64, 35.13. HRMS(MALDI): calcd. for $C_{39}H_{40}N_5O_5$(M+H^+) 658.3029; found 658.3031.

1-[2′-Deoxy-β-D-ribofuranosyl]-5-[N-(dimethylamino)-methylidine]amino-pyrrolo[2,3-f]Quinazoline (10) ^{1}H NMR ($CDCl_3$,500 MHz):δ 9.07(s,1H),8.85(s,1H),7.47(m,3H),7.34 (d,1H,J=3.5 Hz), 6.43(dd,1H,J=6.5,5.5 Hz), 4.81(m,1H), 4.04(m,1H), 3.92(m,2H), 3.21(s,3H), 3.18(s,3H), 2.57(m,1H), 2.51(m,1H). ^{13}C NMR($CDCl_3$,125 MHz): δ 163.31,160.39,158.89, 148.42,137.20, 123.41, 122.91, 120.85, 117.44, 110.56, 104.02, 86.65, 84.62, 70.58, 62.06, 41.38,40.66,35.35. HRMS(DEI): calcd. for $C_{18}H_{21}N_5O_3$ (M^+) 355.1644; found 355.1655.

1-[2′-Deoxy-3′-O-(2-cyanoethyl-N,N-diisopropylphosphino)-5′-O-(4,4′-dimethyloxytrityl)-β-D-ribofuranosyl]-5-[N-(dimethylamino)methylidine]aminopyrrolo[2,3-f]Quinazoline (11) 425 mg DMTr-protected 2′-deoxyriboside(0.65 mmol) was dissolved in 10 mL anhydrous dichloromethane and 0.17 mL diisopropylethylamine (0.98 mmol). To the solution was added 0.17 mL 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.77 mmol). The mixture was stirred at room temperature under Ar atmosphere for 30 min. It was then directly purified by silica gel chromatography (dichloromethane/methanol=40/1) to give 390 mg light yellowish foam in 70% yield. ^{1}H NMR($CDCl_3$,500 MHz) δ 9.12(s,1H),8.87(s,1H),7.63(m,1H),7.42-7.19(m,12H),6.76(m,4H),6 .47(m,1H),4.75(m,1H),4.26(m,1H),3.74(m,6H),3.63-3.40(m,2H),3.3 8-3.29(m,4H),3.24(s,3H),3.19(s,3H),2.77-2.48(m,4H),1.29-1.10(m ,12H).^{13}C NMR (CDCl3,125 MHz) δ 163.74,160.36,158.42,148.73,144.53, 137.59,135.64,130.08,128.22,127.78,126.80,123.32,122.96,120.98, 117.47, 113.12, 110.76, 104.08, 86.36, 85.42, 84.93, 74.14, 63.58, 58.39,55.17, 45.27, 43.27, 43.12, 41.06, 39.91, 35.14, 24.59, 24.46, 22.94, 20.39, 20.34, 20.21, 20.15. HRMS(MALDI): calcd. for $C_{48}H_{57}N_7O_6P$($M+H^+$) 858.4108; found 858.4074.

Oligonucleotide synthesis and characterization. All oligonucleotides were synthesized by the standard DNA synthesis procedures on an ABI 394 DNA/RNA synthesizer. Deprotection of synthesized oligonucleotides was done by 1 mL concentrated ammonium hydroxide at 55 °C for 14 h. Natural oligonucleotides were purified by Poly-Pak II columns purchased from Glen Research company, following the standard procedure. Oligonucleotides containing unnatural bases were purified by dialysis or Polyacrylamide Gel Electrophoresis (PAGE) or HPLC. All synthesized oligonucleotides were confirmed by mass spectroscopy.

Table S1. Mass spectrometry data for oligonucleotides containing yA deoxyribosides.

	Oligonucleotide[a]	Average MW[b],[c]	MW found
1	5'-T^{y}AT	909.2	909.3
2	5'-^{y}ACGCGCG	2155	2158
3	5'-CTTTTC^{y}ATTCTT	3601	3602
4	5'-AAGAA^{y}AGAAAAG	3794	3794
5	5'-^{y}AT^{y}A^{y}AT^{y}ATT^{y}AT	3270	3271
6	5'-^{y}AT^{y}A^{y}ATTT^{y}A^{y}AT	3270	3271
7	5'-^{y}AT T^{y}A^{y}A^{y}ATT^{y}AT	3270	3269

[a] Entry 1 was purified by dialysis, entries 2-5 were purified by PAGE, entries 6-7 were purified by HPLC; [b] For entry 1, MW is the monoisotopic MW for $M+H^+$; For entries 2-7, MW is calculated for free acid. [c] MW for entry 1 was obtained by ESI mass spectrometry; MW for entries 2-7 was obtained by MALDI mass spectrometry, purified natural oligonucleotide dT_{10} was used as an internal reference.

Thermal denaturation experiments. Duplex DNA solutions were heated to 95 °C for 5 min and annealed by slow cooling (over 2 h to room temperature). The melting studies were carried out in 1 cm path-length quartz cells on a Varian Cary 100 Bio UV-Vis spectrophotometer equipped with a Varian Cary temperature controller. Absorbance was monitored at 260 nm while temperature was raised from 0°C or 5°C. Duplex sequences and conditions were as described in the main paper (Table 1). Total oligonucleotide concentration was 5 µM for entries 16 and 17, and 2 µM for entries 18 and 19.

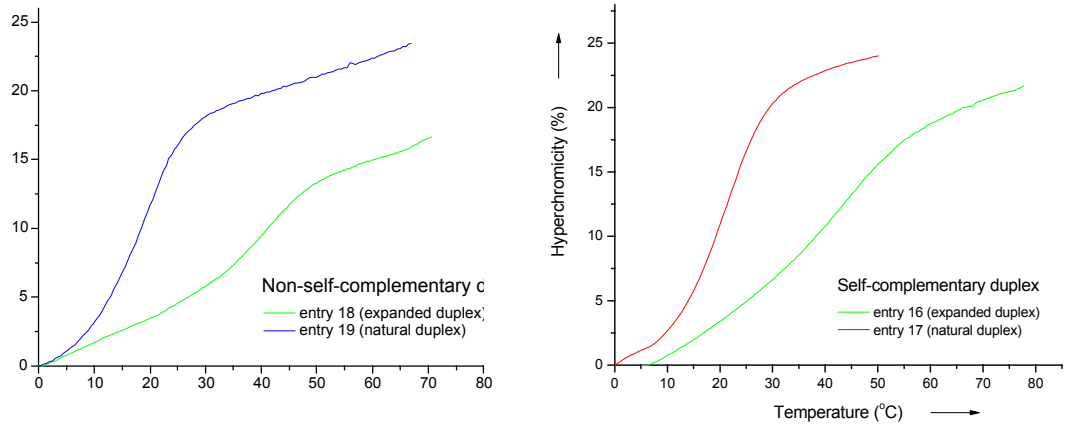

Figure S1. Thermal denaturation curves for expanded yDNA helices and for natural DNA helices of the analogous sequences.

Circular Dichroism spectra. CD spectra were recorded on an *AVIV* CD spectrometer (model: 62A DS) from 320 to 215 nm at 15°C. Data were collected for 2 sec in 1 nm intervals in a 1 cm path-length quartz cell. All samples were in 5 μM total oligonucleotide concentration with 1:1 single strand ratio when paired. The buffer solution contained 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na•PIPES, pH=7.0.

Table S2. Oligonucleotides used in CD spectroscopy studies

	Oligonucleotide
1	5'-ATAATTTAAT
2	5'-ATTAAATTAT
3	5'-^{y}AT^{y}A^{y}ATTT^{y}A^{y}AT
4	5'-^{y}AT T^{y}A^{y}A^{y}AT T^{y}AT

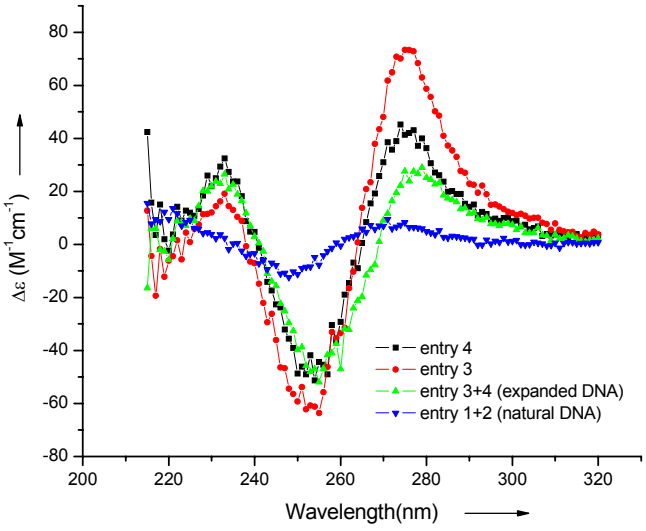

Figure S2. CD spectra for duplex and single-stranded DNA.